African Journal of
Pharmacy and Pharmacology

ABOUT AJPP

The **African Journal of Pharmacy and Pharmacology (AJPP)** is published weekly (one volume per year) by Academic Journals.

African Journal of Pharmacy and Pharmacology (AJPP) is an open access journal that provides rapid publication (weekly) of articles in all areas of Pharmaceutical Science such as Pharmaceutical Microbiology, Pharmaceutical Raw Material Science, Formulations, Molecular modeling, Health sector Reforms, Drug Delivery, Pharmacokinetics and Pharmacodynamics, Pharmacognosy, Social and Administrative Pharmacy, Pharmaceutics and Pharmaceutical Microbiology, Herbal Medicines research, Pharmaceutical Raw Materials development/utilization, Novel drug delivery systems, Polymer/Cosmetic Science, Food/Drug Interaction, Herbal drugs evaluation, Physical Pharmaceutics, Medication management, Cosmetic Science, pharmaceuticals, pharmacology, pharmaceutical research etc. The Journal welcomes the submission of manuscripts that meet the general criteria of significance and scientific excellence. Papers will be published shortly after acceptance. All articles published in AJPP are peer-reviewed.

Contact Us

Editorial Board

African Journal of Pharmacy and Pharmacology

Table of Contents: Volume 12   Number 22   22 July, 2018

ARTICLES

AJ ACADEMIC JOURNALS
expand your knowledge

African Journal of Pharmacy and Pharmacology

Full Length Research Paper

Assessment of antimalarial activity and proteomics analysis of *Dioscorea membranacea* Pierre.

Phunuch Muhamad[1], Luxana Panrit[1], Artitiya Thiengsusuk[1], Wanna Chaijaroenkul[2] and Kesara Na-Bangchang[2]*

[1]Drug Discovery and Development Center, Office of Advanced Science and Technology, Thammasat University (Rangsit Campus), Pathumtani 12121, Thailand.
[2]Center of Excellence in Pharmacology and Molecular Biology of Malaria and Cholangiocarcinoma, Chulabhorn International College of Medicine, Thammasat University, Pathumthani 12121, Thailand.

Multidrug resistance *Plasmodium falciparum* remains a significant global health problem worldwide. New alternative antimalarial drugs are urgently needed. *Dioscorea membranacea* Pierre. is a Thai-medicinal plant that has been shown to exhibit a wide range of pharmacological activities. The study aimed to investigate antimalarial activity and possible protein targets of action of the crude ethanolic extract of the rhizome of this plant. The *in vitro* antimalarial activity expressed as IC_{50} (concentration that inhibits the parasite growth by 50%) of the extract against 3D7 chloroquine-sensitive *P. falciparum* and K1 chloroquine-resistant *P. falciparum* clones were 10.1 (8.8-10.3) and 9.3 (9.17-9.63) μg/ml [median (range)], respectively. The cytotoxicity of against the human fibroblast cell OUMS-36T-1F was 96.4 (96.3-96.5) μg/ml. The selectivity index (SI) for the 3D7 and K1 clones was 9.5 and 10.4, respectively. Preliminary investigation of the protein targets of action in 3D7 *P. falciparum* clone revealed 13 up-regulated protein spots and 14 down-regulated protein spots. For further development of *D. membranacea* Pierre. as a promising antimalarial drug candidate, identification of these proteins by mass spectrometry and investigation of their mode of antimalarial actions are encouraged.

Key words: Malaria, proteomics, *Dioscorea membranacea* Pierre.

INTRODUCTION

Malaria is a vector-borne disease that remains a significant public health problem in tropical and subtropical regions of the world (World Health Organization, 2017). The emergence and spread of multidrug-resistant *Plasmodium falciparum* have become problematic for controlling malaria in most endemic regions of the world including Thailand. Nevertheless,

antimalarial chemotherapy remains the mainstay for controlling malaria in the absence of effective vaccines. There is a pressing need for ongoing drug discovery research that will provide safe, effective, and affordable antimalarial agents. Several approaches have been applied for searching for new antimalarial drugs. Among these is investigation of natural - product-derived

compounds for their potential antimalarial activity. *Dioscorea membranacea* Pierre. locally known in Thai as 'Hua-Kao-Yen-Tai', has long been used as a common ingredient in several Thai medicinal preparations including those for treatment of dermopathy, lymphopathy, inflammation, cancers, neural diseases, and leprosy (Itharat, 2010). The antimalarial activity of this plant has recently been reported by Thiengsusuk et al. (2013). The crude extract of the plant rhizome showed potent antimalarial activity with median IC_{50} (the concentrations that inhibit the parasite growth by 50%) values of less than 10 µg/ml against both K1 (chloroquine resistant) and 3D7 (chloroquine sensitive) *P. falciparum* clones. The objective of the present study was to confirm the antimalarial activity and identify possible protein targets of antimalarial action of the crude ethanolic extract of *D. membranacea* Pierre. (rhizome) using proteomics approach.

MATERIALS AND METHODS

Chemicals and reagents

RPMI, HEPES, and gentamicin were supplied by Gibco BRL Life Technologies (Grand Island, NY, USA). Chloroquine, SYBR Green I and 3-(4,5-dimethyl-2 thiazoyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) were purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA). Ethanol was purchased from Labscan Co. Ltd. (Bangkok, Thailand).

Crude extract of *D. membranacea* Pierre. (rhizome), chemicals, and reagents

The crude extract of *D. membranacea* Pierre. was prepared by maceration method (Itharat et al., 2003). In brief, the plant rhizome was washed, cut into small pieces, air-dried, weighed, and ground into powder. The powder (100 g) was soaked in 300 ml of absolute ethanol for 3 days (1:3 w/v ratio), and the extract was filtered and evaporated under reduced pressure by rotary evaporation. The extract yield (2.93%) was weighed and stored at -20°C until it was used.

Assessment of *in vitro* antimalarial activity of the crude ethanolic extract of *D. membranacea* Pierre.

Two *P. falciparum* clones, that is, 3D7 and K1 were used in the study. The parasites were cultured according to the traditional method of Trager and Jensen (1976). Both were maintained in continuous culture in O+ human erythrocytes suspended in RPMI culture medium supplemented with 10% human B serum and 25 mM HEPES (at 37°C under 5% CO_2, 5% O_2, and 90% N_2 atmosphere). The parasites were synchronized to ring stage *P. falciparum* using 5% sorbitol. Antimalarial activity of the crude ethanolic extract of *D. membranacea* Pierre. (rhizome) was assessed using SYBR Green I assay (Bennett et al., 2004a; Smilkstein et al., 2004). Highly synchronous ring stage parasite was used in each assay. An aliquot of parasite inoculum (50 µl) with 2% parasitemia and 1% hematocrit was added into each well of a 96-well microtiter plate. The 96-well drug plates were dosed with the extract at eight final concentrations as follows: 1.5625, 3.125, 6.25, 12.5, 25, 50, 100, and 200 µg/ml. The experiment was done three

times in triplicate each. Data are presented as median (range) values. The IC_{50} values (concentrations that inhibit the parasite growth by 50%) used as indicators of antimalarial activity were determined from log-dose-response curves using the Calcusyn™ version 1.1 (BioSoft, Cambridge, UK).

Assessment of cytotoxicity of the crude ethanolic extract of *D. membranacea* Pierre.

The normal human embryo fibroblast cell OUMS-36T-1F was purchased from Japanese Collection of Research Bioresources (JCRB) cell bank, Japan. The cell was cultured in RPMI 1640 medium (Gibco Co. Ltd., NY, USA) supplemented with 10% (v/v) heated fetal bovine serum (FBS) and 100 IU/ml of antibiotic-antimycotic solution (Gibco Co. Ltd., NY, USA). The cytotoxicity of the extract was determined by MTT assay (Mosmann, 1983). Briefly, the OUMS-36T-1F cell was seeded onto each well of the 96-well microtiter plate (10,000 cells/well) and incubated for 24 h (37°C, 5% CO_2 atmosphere, and 95% humidity) before exposure to various concentrations of the extract (250, 125, 62.5, 31.25, 15.6, 7.8, 3.9, and 1.95 µg/ml). Following the 48 h incubation, the MTT reagent (20 µl of 5 mg/ml solution; Sigma Co. Ltd., MO, USA) was added into each well, and the plate was further incubated for 4 h. The culture medium of each well was discarded, and DMSO (100 µl) was added, and the plate was incubated at 25°C in the darkroom for 15 min. The absorbance was measured at 570 nm (Varioscan Flash, Thermo, Finland). Cell viability and corresponding IC_{50} was determined using CalcuSyn™ v2.11 software (Biosoft, Cambridge, UK). The experiment was done three times, in triplicate each. Data are presented as median (range) values. The selectivity index (SI) was defined as the ratio of IC_{50} of the crude ethanolic extract of *D. membranacea* Pierre. against malaria in the OUMS-36T-1F cell.

Morphological change of parasite cells following exposure to the crude ethanolic extract of *D. membranacea* Pierre.

Synchronized 3D7 *P. falciparum* was used in the experiment. The parasite was exposed to the crude ethanolic extract of *D. membranacea* Pierre. (rhizome) at the IC_{50} at 37°C under 5% CO_2, 5% O_2, and 90% N_2 atmosphere for 48 h. Blood films were prepared at the following time points: 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, and 48 h and stained with Giemsa (Biotechnical Thai, Bangkok, Thailand). Parasite cell morphology was observed under a light microscope (x100, Olympus, Tokyo, Japan).

Extraction of *P. falciparum* proteins following exposure to the ethanolic extract of *D. membranacea* Pierre.

Synchronized 3D7 *P. falciparum* was exposed to the ethanolic extract of *D. membranacea* Pierre. at the IC_{50} for 24 h. Parasite culture was harvested and the cell pellet was resuspended in 0.15% saponin and incubated on ice for 1 h to lyse red blood cells. The lysate was collected through centrifugation at 13,000 × g for 5 min (4°C) and washed three times with 1 ml of 50 mM Tris pH 7.5 until the supernatant was clear. The parasite pellet was re-suspended in 500 µl of rehydration buffer (8 M urea, 2 M thiourea, 2% CHAPS, 65 mM DTT, 1% ampholyte pH 3-10, and 1x of proteinase inhibitor). The sample was vortexed and sonicated on ice four times, 6 s each (21% amplitude, 6 s, interspersed with 9 s), followed by centrifugation at 13,000 × g for 1 h (4°C). Protein concentration was measured using Bradford reagent (BioRad Co. Ltd., California, USA) and the supernatant was subjected to analysis by 2-dimensional gel electrophoresis (2-DE). The experiment was done four times each.

Table 1. Antimalarial activity and cytotoxicity of the crude ethanolic extract of *Dioscorea membranacea* Pierre.

Sample	Median IC$_{50}$ (range, μg/ml)			Selectivity index	
	3D7	K1	OUMS-36T-1F	3D7	K1
Dioscorea membranacea Pierre.	10.1 (8.8-10.3)	9.3 (9.17-9.63)	96.4 (96.3-96.5)	9.5	10.4
Chloroquine	0.005 (0.005-0.006)	0.066 (0.056-0.071)	ND	ND	ND

Data are presented as IC$_{50}$ values of triplicate experiments, triplicate for each experiment. *ND: Not done.

2-Dimensional gel electrophoresis

The extract of the parasite protein (100 μg) was mixed with rehydration buffer (8 M urea, 1%CHAP, 15 mM dithiothreitol, and 0.001% bromophenol blue) to prepare protein mixture (125 μl) and applied onto 7 cm IPG strips (non-linear) with a pH range of 4 to 7 in an isoelectric focusing (IEF) system (PROTEAN® i12™ IEF Cell, BioRad Co. Ltd., California, USA). The IEF was initially performed at 250 v for 15 min, followed by 4,000 v for 1 h, and 4000 to 20,000 v-h. The focused strips were equilibrated in equilibration solution I (10 ml of 50 mM Tris-HCl, pH 8.8, 6 M urea, 30% glycerol, and 2% SDS) containing the reducing agent DTT (100 mg) for 10 min, followed by equilibration solution II (5 ml) containing iodoacetamide (450 mg) for additional 10 min. Finally, the strips were equilibrated with 1x electrode buffer (pH 8.3) for 10 min and loaded onto 12% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) for second dimension separation. The gels were run on 1x electrode buffer (pH 8.3). The gels were fixed and stained with silver stain (BioRad Co. Ltd., California, USA) according to the manufacturer's recommendation. The 2-DE gel images were scanned and analyzed using PDQuest™ software (BioRad Co. Ltd., California, USA). At least four independent gels were analyzed for each sample group.

RESULTS

The median (range) IC$_{50}$ values of the ethanolic extract of *D. membranacea* Pierre. for the 3D7 and K1 *P. falciparum* clones were 10.1 (8.8-10.3) and 9.3 (9.17-9.63) μg/ml, respectively. The cytotoxicity assay was performed using human fibroblast cell line (OUMS-36T-1F) to evaluate the selectivity of antimalarial activity. The median (range) IC$_{50}$ for cytotoxicity result of the extract was 96.4 (96.3-96.5) μg/ml. The selectivity index of the extract for 3D7 and K1 *P. falciparum* was 9.5 and 10.4, respectively (Table 1).

The morphological change of 3D7 *P. falciparum* following exposure to the ethanolic extract of *D. membranacea* Pierre. at the IC$_{50}$ was observed during the period of 4 to 48 h (Figure 1). During 4 to 12 h, the morphology of the exposed parasites was similar to the control parasite (exposed to 50% ethanol). A ring-like cytoplasm enclosing a vacuole with a dot of round nuclear chromatin projecting from the cytoplasm was observed. The stippling of Maurer's dots was found, and the cytoplasm of the parasite was thickening. The trophozoite stage showed approximately half the diameter (0.8 to 1.1 μm) of the infected red cell. During the period of 16 to 24 h, the control parasite developed

to late and mature trophozoite stages. Moreover, the nuclear chromatin mass was more conspicuous but remained undivided. Following exposure to the extract on the other hand, a marked change in morphology was observed in about 50% of the parasite. The parasite was slowly grown, and the cytoplasm was shrink and dense as compared to control. This alteration was more prominent after 24 h of exposure; the trophozoites appeared relatively smaller as compared to the control with the diameter being reduced to approximately one fourth to one third. During the period of 28 to 48 h, the control parasite developed to early and mature schizont stages. The nuclear chromatin was divided and developed to 24 to 28 merozoites. Thereafter, the mature schizonts began to rupture and re-infected new red blood cells. The growth of about 50% of the exposed parasites was however arrested in the trophozoite stage and finally died.

Based on the morphological changes observed with the 3D7 *P. falciparum* clone following exposure to the extract, suitable time for proteomics analysis was 24 h. The protein patterns of non-exposed and extract exposed parasites are as shown in Figures 2 and 3, respectively. The protein spots were observed following separation with IPG at a pH range of 4 to 7. A total number of approximately 83 and 257 protein spots were separated throughout the gel from the control and exposed parasites, respectively. The patterns of protein spots separated from the control and exposed parasites were found to be similar, of which only 56 protein spots were likely to be the same essential proteins. On the other hand, 27 spots from control and 201 spots from exposed parasite were unmatched spots (Figure 4). The increase in protein expression of about 2- to 38-fold when compared with the control was observed in a total of 13 protein spots extracted from the exposed parasites (Table 2). The decrease in the expression to about 2 to 47% (ratio of exposed/control range from 0.002 to 0.47) of the exposed parasite was observed with about 14 protein spots (Table 3).

DISCUSSION

The antimalarial activity of the crude ethanolic extract of *D. membranacea* Pierre. rhizome was evaluated in 3D7 chloroquine-sensitive and K1 chloroquine-resistant *P.*
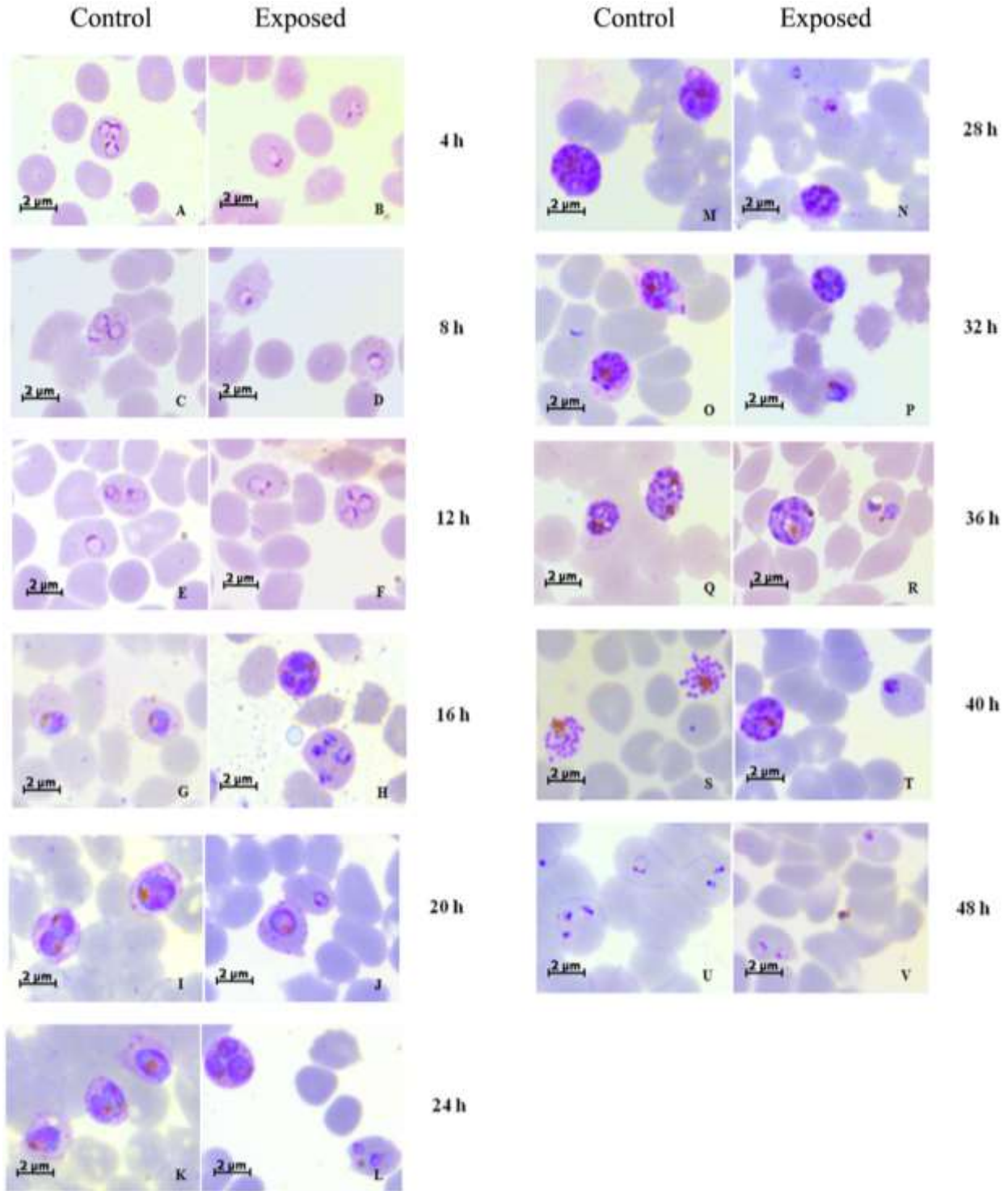
Figure 1. Giemsa-stained thin blood films of 3D7 *P. falciparum* exposed to the ethanolic extract of *Dioscorea membranacea* Pierre. in comparison with control parasite (exposed to 50% ethanol) during the period of 4 to 48 h.

falciparum clones *in vitro.* Results (IC_{50} values of 10.1 and 9.3 µg/ml against 3D7 and K1 clones, respectively) are in agreement with that previously reported (IC_{50} values of 6.2 and 5.1 µg/ml against 3D7 and K1 clones, respectively) (Thiengsusuk et al., 2013). According to the criteria proposed by Rasoanaivo et al. (2004), the IC_{50} of
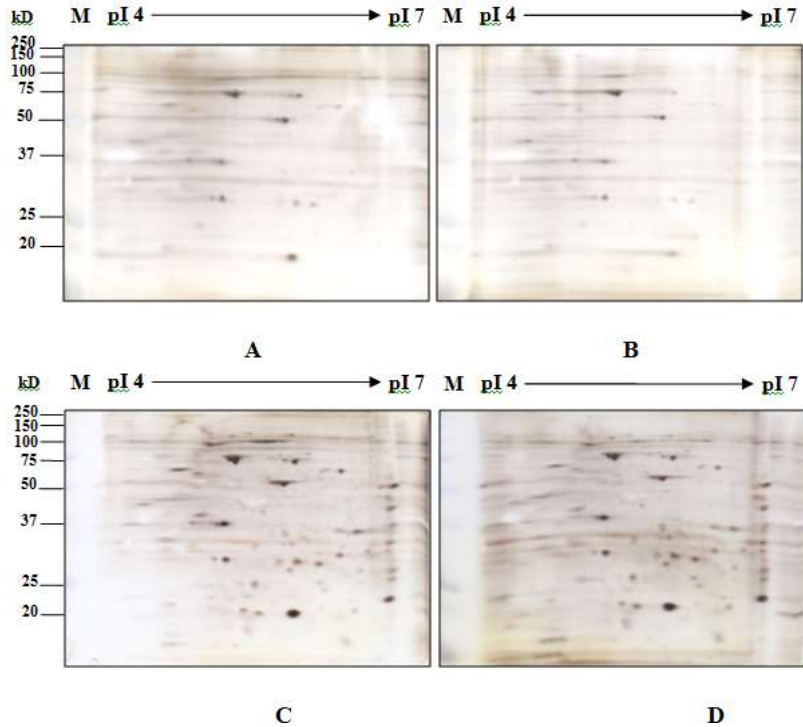
Figure 2. Silver-stained protein spots separated by two-dimensional gel electrophoresis over the pH range 4 to 7, from the control (exposed to 50% ethanol) 3D7 *P. falciparum* clone following a 24-h exposure. Lane M represents marker with the molecular weight indicated at the left.
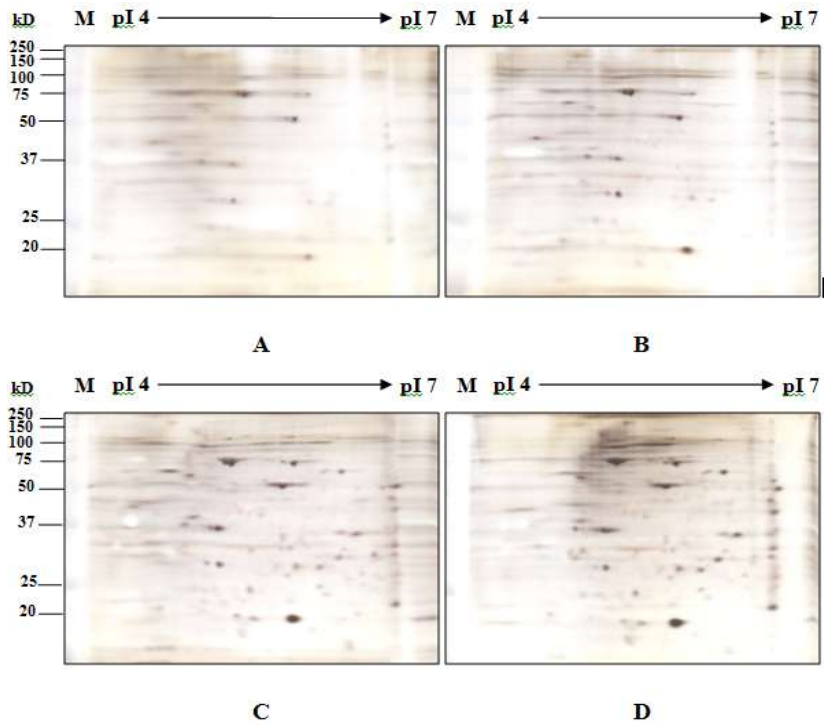
Figure 3. Silver-stained protein spots separated by two-dimensional gel electrophoresis over the pH range 4 to 7, from the exposed-3D7 *P. falciparum* clone following a 24-h exposure. Lane M represents marker with the molecular weight indicated at the left.

Match symbols (matched:56 unmatched:27)
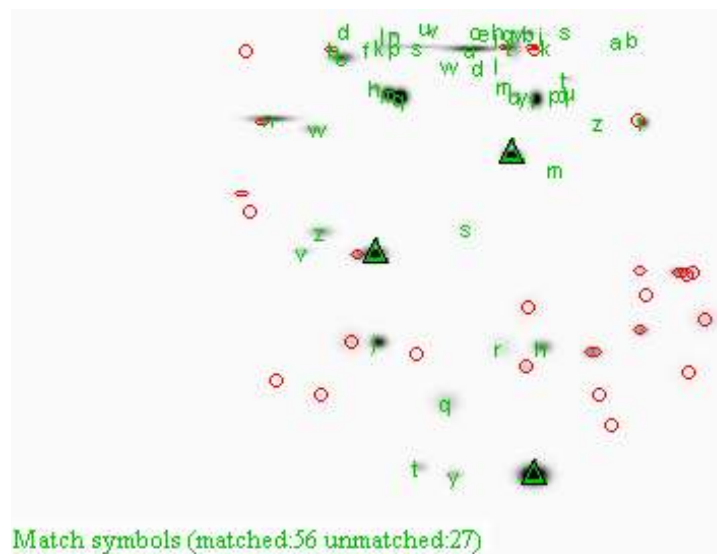
Figure 4. Comparison of silver-stained protein spots separated by two-dimension gel electrophoresis over the pH range of 4 to 7 in the exposed and control (exposed to 50% ethanol) 3D7 of *P. falciparum*. The spots were identified by PDQuest™ (Bio-Rad, the USA). The green spots represent the matched spots and the red spots represent the unmatched spots.

Table 2. Comparison of protein spots detected from the extract of 3D7 *P. falciparum* separated by 2-DE following exposure to the ethanolic extract of *Dioscorea membranacea* Pierre. at the IC_{50} level with at least 2-fold increase in density compared with control.

Spot ID	Protein spot density		The ratio of spot density [Exposed/Control]
	Exposed	Nonexposed	
SSP2603	2198.70	220.80	9.96
SSP2604	10738.70	4210.75	2.55
SSP3201	4106.80	1678.10	2.45
SSP4401	2773.10	1035.10	2.68
SSP5303	6200.50	254.40	24.37
SSP5401	453.80	197.80	2.29
SSP6001	146833.95	52880.70	2.78
SSP6201	5544.20	142.60	38.88
SSP7301	3150.55	623.80	5.05
SSP7303	2129.30	1062.30	2.00
SSP7401	7228.80	1132.10	6.39

The experiment was done four times each.

potential antimalarials from the natural products should be at least 10 μg/ml. The results confirm potent antimalarial activity of ethanolic extract of *D. membranacea* Pierre against *P. falciparum*. The selectivity of the extract against both parasite clones as compared to human normal cell was relatively high (about 10 times). In the previous study, SI of the extract was reported to be 24.2 and 29.4 for 3D7 and K1, respectively. The discrepancy could be due to the difference in sensitivity of the parasite clones to different human cell lines. Human renal epithelium cell was used in the previous study, whereas fibroblast cell line was used in the present study (Thiengsusuk et al., 2013). To further investigate for the time- and stage-specific antimalarial action, the morphological changes of 3D7 *P. falciparum* exposed to the ethanolic extract of *D. membranacea* Pierre. were observed in comparison with the control (exposed to 50% ethanol) parasite during the period of 4 to 48 h. The difference in morphology was observed in the exposed parasite starting from 16 h and

Table 3. Comparison of protein spots detected from the extract of 3D7 *P. falciparum* separated by 2-DE following exposure to the ethanolic extract of *Dioscorea membranacea* Pierre. at the IC$_{50}$ level with at least 2-fold decrease in density compared with control.

Spot ID	Protein spot density		The ratio of spot density [Exposed/Control]
	Exposed	Nonexposed	
1005	1097044	937120	1.17
1006	1031071	642015.9	1.61
1102	957180.4	3241389	0.30
2006	858408.8	1225975.1	0.70
2007	638622.9	32429.7	19.69
2008	513838.9	902078.9	0.57
2009	340800	511576.9	0.67
2106	306116.8	728049.3	0.42
2107	258182.8	1072162.8	0.24
2108	198814.9	308001.8	0.65
2109	122522.1	170356.2	0.72

The experiment was done four times each.

was seen at 24 h. This indicates that the plant was likely to act on the growing trophozoite stage (12 to 24 h).

More than 40 Thai medicinal plants have been screened for antimalarial activity (Gale et al., 2007; Pinmai et al., 2010; Thiengsusuk et al., 2013). The promising plants include *Phyllanthus emblica*, *Terminalia chebula*, *Terminalia bellerica* (Pinmai et al., 2010), *Plumbago indica* Linn. (root), *Garcinia mangostana* Linn. (pericarp), *Dracaena loureiri* Gagnep. (stem), *Piper chaba* Hunt. (fruit), *Myristica fragrans* Houtt. (seed), *Kaempferia galangal* Linn. (leave), *Artemisia annua* Linn. (rhizome), and *D. membranacea* Pierre. (rhizome) (Thiengsusuk et al., 2013). The potency of antimalarial activity (IC$_{50}$) of these plants range from 3 to 15 µg/ml. Maceration was applied as the method for extraction of all plants but with different solvents. In the study reported by Pinmai et al. (2010), water was used as the extraction solvent, whereas for those reported by Gale et al. (2007) and Thiengsusuk et al., (2013), ethanol was used as the extraction solvent. The selection of solvents in all studies follows the practical use in traditional medicine.

Apart from antimalarial activity, the ethanolic extract of *D. membranacea* Pierre. rhizome has been report to exhibit a wide range of pharmacological activities such as cytotoxic against various cancer cell lines (COR-L23, LS-174T, MCF-7, and SVK-14) (Itharat et al., 2003), anti-HIV-1 protease- and HIV-1 integrase activities (Tewtrakul et al., 2006), immunomodulatory activity (Panthong et al., 2014; Tewtrakul and Itharat, 2006). Dioscorealides, dioscoreanone discorealide B, dihydrophenanthrene are bioactive compounds isolated from its rhizome, which were responsible for these pharmacological activities (Thongdeeying et al., 2016; Itharat and Hiansai, 2012; Saekoo et al., 2010).

The proteomics analysis is one of the commonly used approaches for protein identification that has been applied in various types of research. These include the identification of biomarkers of disease pathogenesis, protein targets of action of new drug candidates, protein targets of drug resistance, and vaccine development (Cooper and Carucci, 2004; Moorthy et al., 2004; Singh et al., 2009). Proteomics is a large-scale study of proteome which includes their structures and functions. These proteins are responsible for the phenotype of cells responded to the environment and therefore, is much more complicated than the genomics approach due to a marked difference in protein types and quantity from cell to cell over time. In addition, the obstacles of proteomics research in malaria are difficulty in protein extraction and protein solubility (Rungsihirunrat et al., 2012). The commonly used technique to study proteomics is the two-dimensional gel electrophoresis (2-DE), which separates solubilized proteins in the first dimension according to their charges by using isoelectric focusing, followed by the second dimension which separates proteins according to their molecular weight (Bernard et al., 2004b; Gevaert and Vandekerckhove, 2000). In this study, insolubility of malarial membrane proteins resulted in low efficiency of separation of the protein spots. It is noted that the proteomics was performed only in the 3D7 *P. falciparum* clone as the change can be observed in the parasite carrying natural gene (wild-type). This ensures that the change that occurred was the effect of the extract instead of the mutation of parasite gene. The 2DE was used to analyze the expression and patterns of proteins separated from 3D7 *P. falciparum* clone following exposure to the extract for 24 h in comparison with the control parasite. The efficiency of protein separation was improved by cleaning up the insoluble parasite proteins by centrifugation parasite suspension (4°C) at 11,300 $\times$ *g* for 1 h. Abundant malaria proteins were separated at the pH gradients ranging from 4 to 7. Significantly higher

number of protein spots (201 spots) was separated from the exposed as compared to the non-exposed parasite. This suggests that only a few or none of the protein was expressed during normal condition but was over-expressed following exposure of the parasite to the extract. Among the protein spots separated from parasite exposed to the plant extract, 27 protein spots were unmatched protein spots, of which 13 and 14 spots showed up- and down-regulation of expression, respectively (at least 2-fold difference in expression when compared with the control). Unfortunately the protein identification was not successful, these proteins showed hypothetical proteins with unknown function. The previous report of proteomics study from ethanolic extracts of *G. mangostana* against *P. falciparum* had been reported. The IPG strip in this study was in a wider range (pH 3 to 10) than the present study (IPG strip pH 4 to 7). This difference was due to the result of primary proteomics work, most protein spots of our study were localized at pH 4 to 7. Therefore, we designed to use the IPG strip pH 4 to 7. In this present work, only 3D7 *P. falciparum* clone was used in proteomic study, because the native genetic background of this clone will not interfere with the changed of protein pattern.

Conclusion

For further development of *D. membranacea* Pierre. as a promising antimalarial drug candidate, identification of these proteins by mass spectrometry and investigation of their mode of antimalarial actions are encouraged.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests

REFERENCES

Bennett TN, Paguio M, Gligorijevic B, Seudieu C, Kosar AD, Davidson E, Roepe PD (2004a). Novel, rapid, and inexpensive cell-based quantification of antimalarial drug efficacy. Antimicrobial Agents and Chemotherapy 48(5):1807-1810.

Bernard KR, Jonscher KR, Resing KA, Ahn NG (2004b). Methods in functional proteomics: two-dimensional polyacrylamide gel electrophoresis with immobilized pH gradients, in-gel digestion and identification of proteins by mass spectrometry. Methods in Molecular Biology 250:263-282.

Cooper RA, Carucci DJ (2004). Proteomic approaches to studying drug targets and resistance in Plasmodium. Current Drug Targets. Infectious Disorders 4:41-51.

Gale GA, Kirtikara K, Pittayakhajonwut P, Sivichai S, Thebtaranonth Y, Thongpanchang C, Vichai V (2007). In search of cyclooxygenase inhibitors, anti-Mycobacterium tuberculosis and anti-malarial drugs from Thai flora and microbes. Pharmacology and Therapeutics 115(3):307-351.

Gevaert K, Vandekerckhove J (2000). Protein identification methods in proteomics. Electrophoresis 21(6):1145-1154.

Itharat A (2010). Comparative biological activities of five Thai medicinal plants called Hua-Khao-Yen. Thai Journal of Pharmacology 32(1):327-331.

Itharat A, Hiransai P (2012). Dioscoreanone suppresses LPS-induced nitric oxide production and inflammatory cytokine expression in RAW 264.7 macrophages by NF-κB and ERK1/2 signaling transduction. Journal of Cellular Biochemistry 113(11):3427-3435.

Itharat A, Plubrukarn A, Kongsaeree P, Bui T, Keawpradub N, Houghton PJ (2003). Dioscorealides and dioscoreanone, novel cytotoxic naphthofuranoxepins, and 1,4-phenanthraquinone from *Dioscorea membranacea* Pierre. Organic Letter 5(16):2879-2882.

Moorthy VS, Good MF, Hill AV (2004). Malaria vaccine developments. Lancet 363:150-156.

Mosmann T (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of Immunological Methods 65:55-63.

Panthong S, Ruangnoo S, Thongdeeying P, Sriwanthana B, Itharat A (2014). Immunomodulatory activity of *Dioscorea membranacea* Pierre rhizomes and of its main active constituent Dioscorealide B. BMC Complementary and Alternative Medicine 14:403.

Pinmai K, Hiriote W, Soonthornchareonnon N, Jongsakul K, Sireeratawong S, Tor-Udom S (2010). *In vitro* and *in vivo* antiplasmodial activity and cytotoxicity of water extracts of *Phyllanthus emblica*, *Terminalia chebula*, and *Terminalia bellerica*. Journal of the Medical Association of Thailand 93(Suppl 7):S120-6.

Rasoanaivo P, Deharo E, Ratsimamanga-Urverg S, Frappier F (2004). Guidelines for the Nonclinical Evaluation of the Efficacy of Traditional Antimalarials. In: Traditional Medicinal Plants and Malaria, CRC Press.

Rungsihirunrat K, Chaijaroenkul W, Siripoon N, Seugorn A, Thaithong S, Na-Bangchang K (2012). Comparison of protein patterns between *Plasmodium falciparum* mutant clone T9/94-M1-1(b3) induced by pyrimethamine and the original parent clone T9/94. Asian Pacific Journal of Tropical Biomedicine 2:66-69.

Saekoo J, Dechsukum C, Graidist P, Itharat A (2010). Cytotoxic effect and its mechanism of dioscorealide B from Dioscorea membranacea against breast cancer cells. Journal of the Medical Association of Thailand 93 (12):S277-82.

Singh M, Mukherjee P, Narayanasamy K, Arora R, Sen SD, Gupta S, Natarajan K, Malhotra P (2009). Proteome analysis of *Plasmodium falciparum* extracellular secretory antigens at asexual blood stages reveals a cohort of proteins with possible roles in immune modulation and signaling. The Molecular and Cellular Proteomics 8(9):2102-2118.

Smilkstein M, Sriwilaijaroen N, Kelly JX, Wilairat P, Riscoe M (2004). Simple and inexpensive fluorescence-based technique for high-throughput antimalarial drug screening. Antimicrobial Agents and Chemotherapy 48(5):1803-1806.

Tewtrakul S, Itharat A (2006). Anti-allergic substances from the rhizomes of Dioscorea membranacea. Bioorganic and Medicinal Chemistry 14(24):8707-8711.

Tewtrakul S, Itharat A, Rattanasuwan P (2006). Anti-HIV-1 protease- and HIV-1 integrase activities of Thai medicinal plants known as Hua-Khao-Yen. Journal of Ethnopharmacology 105(1-2):312-315.

Thiengsusuk A, Chaijaroenkul W, Na-Bangchang K (2013). Antimalarial activities of medicinal plants and herbal formulations used in Thai traditional medicine. Parasitology Research 112(4):1475-1481.

Thongdeeying P, Itharat A, Umehara K, Ruangnoo S (2016). A novel steroid and cytotoxic constituents from *Dioscorea membranacea* Pierre against hepatocellular carcinoma and cholangiocarcinoma cells. Journal of Ethnopharmacology 194:91-97.

Trager W, Jensen JB (1976). Human malaria parasites in continuous culture. Science 193:673-675.

World Health Organization (2017). World malaria report 2017 Geneva: World Health Organization. Available at: www.who.int/malaria/publications/world-malaria-report-2017/en/.

Full Length Research Paper

Ethnomedicinal plants used for the treatment of gastro-intestinal parasitic diseases in human in Yeki district, Southwest Ethiopia

Belachew Garedew[*] and Dagne Abebe

Department of Biology, College of Natural and Computational Sciences, Wolkite University, P. O. Box 07, Wolkite, Ethiopia.

The use of medicinal plants plays a major role in the primary health care of human beings in Ethiopia. A study was carried out to document ethnomedicinal plants used for the treatment of gastrointestinal parasitic diseases of human in Yeki district, southwest Ethiopia. The key informants were selected using purposive sampling method. The information was obtained from 26 informants and 8 herbalists through the use of a semi-structured interview and observation on the habitat of medicinal plants. The collected data were analyzed using excel spread sheet. The survey revealed that 29 plant species belonging to 20 families were identified, and they were used for the treatment of gastrointestinal parasitic diseases of human. Among different intestinal parasite studied, protozoa (Amoebiasis) contributed the highest prevalence (48.28%) which infects children frequently. Lamiaceae (13.79%) species was the most frequently used plant family. Leaves were the most frequently used plant parts, constituting 51.72% followed by bark, seed, and fruit each with 6.89%. The remedies were prepared crushing single plant (62.5%) and in few cases mixtures of different plants (37.5%). Plants like *Maytenus arbutifolia* and *Pycnostachys meyeri* were approved by most of the healers which was used to control parasites. The collated data analysis revealed that a protozoan parasite infection is the most prevalence in the area due to less personal and environmental hygiene. The study revealed that information on medicinal plants was mostly confined to older people, and there is danger that this knowledge can be lost before being passed on to next generations. Therefore, there is an urgent need to document information on these plant species so that the future generation can benefit from it. Further investigation should be carried out to validate the efficacy and safety of the aforementioned plants so as to provide cheap alternative ways of controlling parasites.

Key words: Ethnomedicine, gastrointestinal parasite, helminthes, medicinal plants, protozoa.

INTRODUCTION

Traditional medicinal plant is used for the treatment of human ailments throughout the world's primary health care, e.g, human intestinal parasitic diseases by the help of indigenous medical practitioners. There has been an

increase worldwide in the realization of the use of medicinal plants in various traditional health systems of developing countries (Mirutse et al., 2009; Yalew et al., 2012; Tolossa et al., 2013).

In the history of Ethiopia, the use of traditional medicinal plants for treating different human ailments can be confirmed in all parts of the country. The plant remedies are still the most important and sometimes the only source of therapeutics for nearly 80% of the population in Ethiopia, especially rural populations (Lulekal et al., 2008). The collection and processing of medicinal plants provide employment and income opportunities for a large number of people in rural areas (Ngarivhume et al., 2015). The importance of traditional medicinal plants in conservation of biological diversity merits the attention it has gotten worldwide.

Gastrointestinal parasitic infections caused by intestinal protozoa and helminths are among the most common human infections throughout the world especially high prevalence in poor and socio-economically deprived communities in the tropical and subtropical countries including Ethiopia. Parasitic infections due to protozoa and helminthes are responsible for substantial morbidity and mortality worldwide especially children in developing countries (Utzinger et al., 1999; Norhayati et al., 2003; Stepek et al., 2006; Haque, 2007; Ukwubile, 2012; Speich et al., 2013; Traore et al., 2013; Abdullah et al., 2016).

The intestinal parasitic infections are transmitted mainly through fecal contamination of food or water. These are the most frequent in areas where sanitation and hygiene are poor. The most common gastrointestinal parasites that cause frequent diseases/infections on human are protozoa (amoebiasis, giardiasis) and helminthes. Ascariasis, taeniasis (tapeworms), hookworm infection trichuriasis and etc. are among the top ten most common infections in the world (Norhayati et al., 2003; Corrigan et al., 2011). These infections continue to be a global health challenge, particularly among children in poor communities in developing countries. Epidemiological assessments of these infections have traditionally focused on estimating the number of infections (prevalence), which occur worldwide. Unfortunately, rural populations have limited access to health services and anti-parasitic substances used for gastrointestinal parasites control, due to poverty and inaccessibility. On the other hand, most of the existing drugs produce side effects such as abdominal pain, loss of appetite, nausea, vomiting, head ache and diarrhea. Therefore, these people are closely associated with medicinal plants to treat the intestinal parasite infection.

According to Lulekal et al. (2008) and Kefalew (2015), there is a considerable global interest in tapping the accumulated knowledge of traditional medicine and therefore, researches are being carried out in the district region with the aim of increasing the use of traditional medicine to the welfare of the human population. In addition, the increase in population growth rate would result in the intensification of agriculture in marginal areas which would lead to deforestation with decrease in number or loss of medicinal plants in the wild (Pankhurst, 2001). Therefore, the study was carried out to document ethnomedicinal plants used for the treatment of gastrointestinal parasitic diseases in human in Yeki district, southwest Ethiopia and for sustainable conservation of these species.

METHODOLOGY

Study area

The survey was conducted in Yeki District, Sheka Zone, Southwest Ethiopia which was organized by 23 official Kebeles. The area is located at 611 km southwest of Addis Ababa. Geographically, the district lies between 7°12' to 7°43' W latitude and 35°32' to 35°75' E longitude. The altitudinal range of the district falls between 1001 to 2007 m above sea level, and it receives high amount of rainfall with mean average of 1591 mm annually and recorded high rainfall from April to September. Moist evergreen vegetation is the most popular in the area which is conducive for diverse medicinal plants. It is also conducive for cultivation of cash crops such as coffee, ginger, turmeric, piper and others (Yeki Woreda Administration office, 2014). Based on the 2007 Census conducted by the CSA of Ethiopia, this district has a total population of 134,519, of whom 68,895 are men and 65,624 women; 24,829 or 18.46% of its population are urban dwellers. The majority of the inhabitants practiced Ethiopian Orthodox Christianity, with 44.85% of the population reporting their belief, 29.8% were Protestants, 21.66% were Muslim, and 1.99% practiced traditional beliefs. The six largest ethnic groups reported in this Woreda were the Kafficho (29.78%), the Amhara (29.48%), the Oromo (11.67%), the Mocha (7.45%), the Bench (7.33%), and the Sheko (7.26%); all other ethnic groups makes up 7.03% of the population (CSA, 2007) (Figure 1).

Site selection and informant determination

The compiled ethnomedicinal data were gotten from Yeki district, Sheka Zone Southwest Ethiopia in 2017. The study sites were selected purposively from the district of 23 Kebeles/Villages (that is, Gobito, Adisalem, Qorcha and Fide). This sampling technique was first made with the help of a district agricultural office to collate the detail information about herbalist found in the district. All traditional healers or herbalists were incorporated based on the guidance and information of elder people in the locality. The local herbalists and elder informants were selected using both purposive and random sampling from the district to gather anti intestinal parasitic plants common in the locality. A total of 8 herbalists (2 healers from each village) and 26 key informants (approximately 6 elder informants from each village) were gotten. Then the researchers approached the herbalist and elder informants to collect the information on the medicinal plants used to treat gastrointestinal parasite diseases of human. Hence, almost all the interviewed herbalists were volunteers for giving all possible information and trends for treatment of gastrointestinal parasitic diseases effectively in the village.

Data collection method

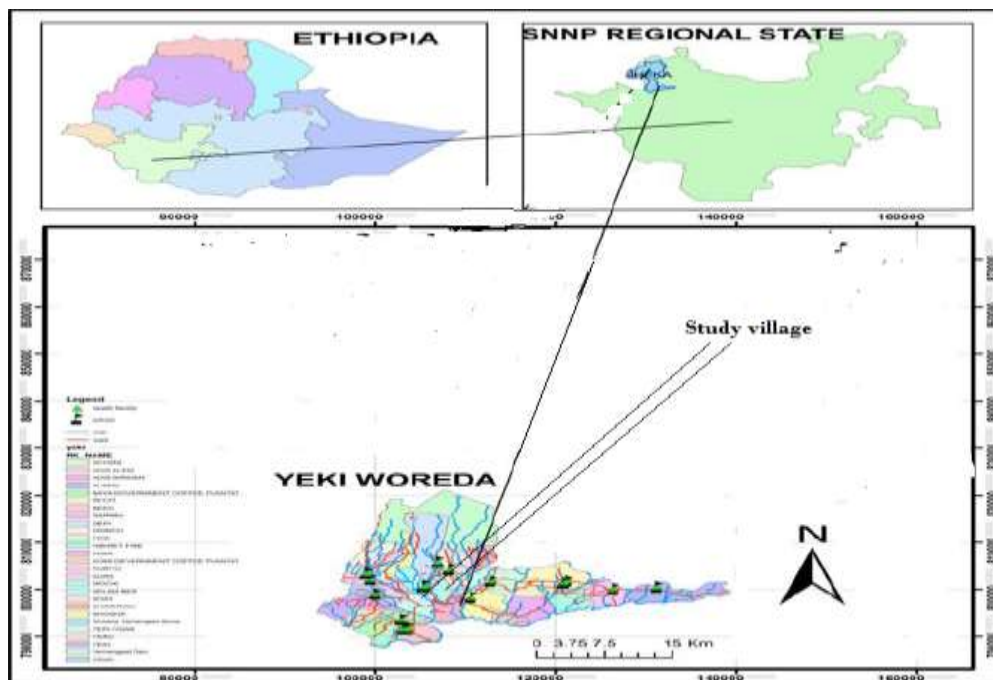Based on the preliminary survey of the site, the traditional healers

Figure1. Map of study area

were able to get at least two round of contact detail information from the community. In order to generate valid and extensive data, the study employed a combination of different method of approaches. These methods were questionnaires, semi-structured interview, focus group discussion, case study, field observation, identification of medicinal plants and informant consensus. The study was carried out to evaluate people's knowledge and their management practices on the valuable medicinal plants used for the treatment of intestinal parasitic infections and economic uses of these plants.

Plant collection and identification

Plant specimens were collected during guided field work with the informants and traditional healers. At times, the field activities included taking notes on plants and the associated indigenous knowledge with preliminary identification of the plants to family and sometimes to species levels. For further information, photograph of the medicinal plants and other important activities of herbalists during remedy preparation were recorded in the field. The plant specimens were pressed, dried and identified at the National Herbarium of Ethiopia using voucher specimens and flora of Ethiopia and Eritrea.

Data analysis

The collected ethnobotanical data reported medicinal plants used for gastrointestinal parasite diseases, and associated indigenous knowledge was entered into Excel spreadsheet and summarized using descriptive statistics. The spreadsheet data filter facility was employed to determine frequencies and percentage of citations by healers so as to identify the most common parts used and route of administration, and habit of medicinal plants preferred for the

treatment of liver problem throughout the country. The results were presented using pie charts, bar chart and tables.

Informant consensus factor (ICF), preference ranking and fidelity level (FL)

The diseases and remedies reported were grouped into categories based on the distribution in the district. The Informant consensus factor (ICF) was calculated for each category to identify the agreements of the informants on reported cures for the group of ailments. The ICF was calculated as follows:

$$ICF = \frac{Nur - Nt}{Nur - 1}$$

Where:

ICF: Informant consensus factor
Nur: number of use citation
Nt: number of species used.

Fidelity level (FL) was calculated to determine the percentage of informants that reported the uses of a medicinal plant as a remedy for the same major intestinal ailment using the formula, FLI = Ip/Iu × 100, where Ip is the number of informants who independently indicate the use of a species for the same major intestinal ailment and Iu the total number of informants who mention the plant for any major intestinal ailment. Preference ranking on plants that were reported by 5 and above informants that was used as a treatment for multiple diseases was conducted. Four informants, one from each village based on the number of medicinal plants reported by each informant, were selected to rank the plants. The informants were briefed on the marking of the plants that the most preferred was given the highest points (1) and least preferred was given the lowest point (6).

RESULT AND DISCUSSIONS

General information of informants

In this survey, a total of 34 persons (26 informants and 8 traditional healers) were involved for reliable data collection in Yeki district of four villages. Most of the informants found in the study area were males (88.24%) because the traditional knowledge of practicing medicinal plants as healers was transmitted from father to the elder son. Therefore, females could not be the healer or practitioners however; four of the elder female informants were selected for approving the practice in the locality. Most of the respondents were between the age of 55 and 75 years old (93.6%). The person in this age group is more experienced and knowledgeable as compare to the younger age. Orthodox religion and informant job account for higher percentage of the study participants.

Prevalence of gastrointestinal parasite diseases and signs

Among the common intestinal parasites affecting human wellbeing were protozoa (48.28%) followed by ascaris (20.69%) (Table 1). Therefore, the study revealed that the most prevalent parasitic diseases in the area were protozoal such as amobia and giardia followed by ascaris. This prevalence is seen because of lack of sanitation in the area.

Similarly, the case is described in some local communities of the world that are exposed to parasitic diseases (Speich et al., 2013; Saqur et al., 2017). The major clinical signs as identified by the traditional herbalists considered as being related to gastrointestinal parasites infections in humans are stomach ache, presence of tapeworm segments in the faeces as well as the presence of blood and mucous in the faeces and/or diarrhea for protozoa parasitic infections. In this case, most of the tradisional herbalists used different diagnosis to identify the type of protozoa and helminthes diseases. Based on this, amoebiasis differ from giardiasis infection due to mucus with faeces and the diarrhoea contains mucus and blood. These results are also in line with the findings of Stepek et al. (2006), Haque (2007) and Saqur et al. (2017) which was conducted in different parts of the world. The parasitic diseases are frequently observed in children compared to adult because children play with each other in the dirt without washing their hands except their parents instruct them to wash their hands. Similarly according to the study of Abdullah et al. (2016) and Saqur et al. (2017), children are mostly faced with intestinal parasitic infection.

The most common gastro-intestinal parasite infected age groups of human in the study area were identified by the traditional medical practitioners and key informants. Based on the response, gastrointestinal parasite frequently affects children of ages of 3 to 10 (47.06%) followed by age group 11 to 20 (26.47%) and less than 2 years old (11.76%) (Figure 2). Hence, these age groups are susceptible to develop the parasitic infection compared to other age groups. This finding was also in agreement with the finding of different researchers conducted across the world by which most of children were infected by intestinal parasitic infections (Norhayati et al., 2003; Stepek et al., 2006; Haque, 2007) (Figure 2).

The result showed that diseases that were frequent in the study area have higher informant consensus factor (ICF). Medicinal plants that are effective in treating certain disease and well known by community members also have higher ICF. Intestine worms had the highest ICF value (1) followed by tapeworms (0.75) whereas; protozoa had the lowest ICF value (0.5) (Table 1). Fidelity level (FL) as an estimation healing potential was determined for some medicinal plants.

Accordingly, *Pycnostachys meyeri, Hagenia abyssinica, Ocimum lamifolium* and *Croton macrostachyus* were the plants having the highest level values for their use to treat intestinal parasitic infections and diseases. As the medicinal plants prescribed by informants for the same health problem such as protozoa infections (amoeba), the informants showed preference of one over the other. Preference ranking of six medicinal plants that were reported for treating amoeba was conducted after selecting six key informants. The informants were asked to compare the given medicinal plants based on their efficacy and to give the highest number (1) for the medicinal plant which they thought most effective and the lowest number (6) for the least effective plant. *Maytenus arbutifolia* and *Pycnostachys meyeri* ranked first indicating that it is the most effective in treating protozoa infections (amoeba) followed by *Prunus Africana* and the least effective was *Brucea antidysenterica* (Table 2).

Medicinal plants used

A total of 29 species of medicinal plants used for the treatment of gastrointestinal parasitic diseases was collected and documented from the study area. These identified species were represented with 20 families. From which, family Lamiaceae had the highest species contributing 4 species (13.79%) and closely followed by family Asteraceae with 3 species (10.34%), and Cucurbitaceae, Euphorbiaceae, Rosaceae and Rubiaceae represented each with 2 species (6.89%). The remaining 14 families represented each by 1 species (Figure 3). The numbers of medicinal plants used for treating human ailments and associated ethnomedicinal knowledge has been observed in different regional state of Ethiopia (Lulekal et al., 2008; Tolossa et al., 2013; Kefalew et al., 2015). The existence and utilization of such a large number of medicinal plants by people in the study area indicates that the majority of the people used indigenous

Table 1. Type and informant consensus value category of intestinal diseases in human in Yeki district.

Categories	Species	% of species	No. of use citation	% of all citation	ICF value
Ascaris	6	20.69	17	25	0.69
Intestine parasite infection	4	13.79	7	10.29	0.5
Intestine worms	1	3.45	3	5.88	1
Protozoa	14	48.28	27	39.71	0.5
Tapeworms	4	13.79	13	19.12	0.75
Total	29	100	-	-	-
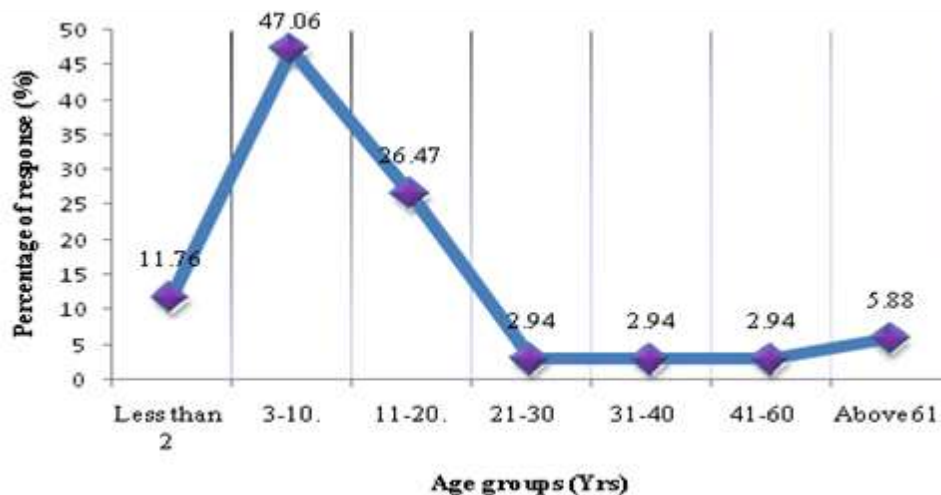
Figure 2. Common age groups infected by intestinal parasite.

Table 2. Preference ranking of six medicinal plants used to treating amoeba disease (R= respondents).

Species	Key respondents						Total	Rank
	R1	R2	R3	R4	R5	R6		
Aeollanthus densiflorus	5	6	4	2	5	5	27	5th
Ajuga integrifolia	4	4	1	5	4	4	22	4th
Brucea antidysenterica	6	5	6	6	5	6	34	6th
Maytenus arbutifolia	3	2	3	3	1	1	13	1st
Prunus africana	1	3	2	4	3	3	16	3rd
Pycnostachys meyeri	2	1	5	1	2	2	13	1st

medicinal practices to treat different health problems. The study shows that the traditional healers of the study area were found to play great roles in the primary healthcare systems of the local people as they were treating resource poor people who had little access and could not afford the cost of modern medications.

Habits of medicinal plants

The assessment on the growth form of the medicinal

plants depicted that herbs constituted the highest number of 11 species (37.93%) collected, and closely followed by tress 7 (24.14%) and 6 species shrub (20.69%) (Figure 4). However, a single plant belongs to epiphytic medicinal plants. This finding show that the most represented life forms of medicinal plants for the treatment of gastrointestinal parasite in the study area was herbs followed by trees. This might show that there is abundance of herbs in the study area because the area is rich with rainfall for nine or more months. This makes the area conducive for the growth of herbs. Therefore, the
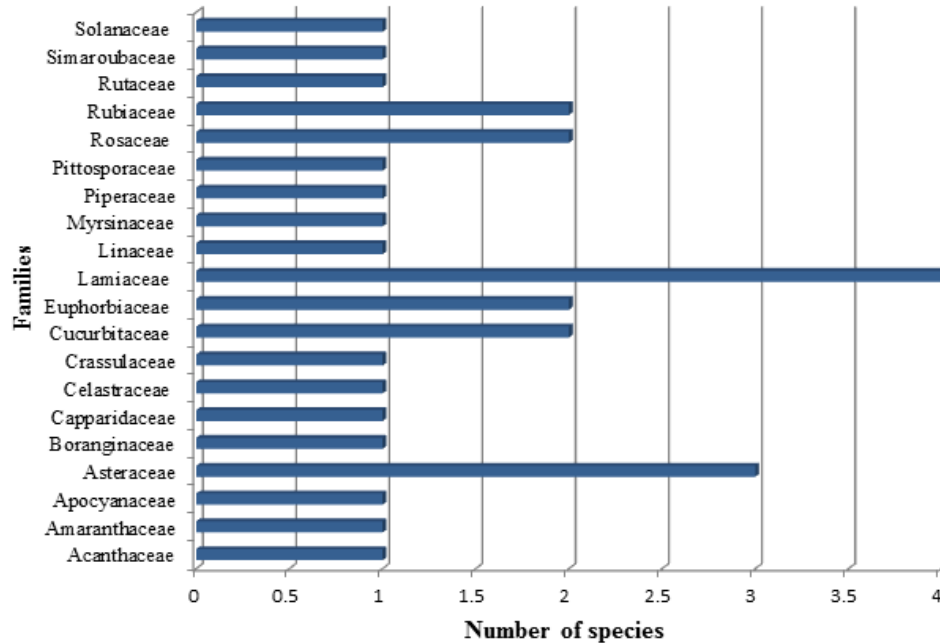
Figure 3. List of families with the species.
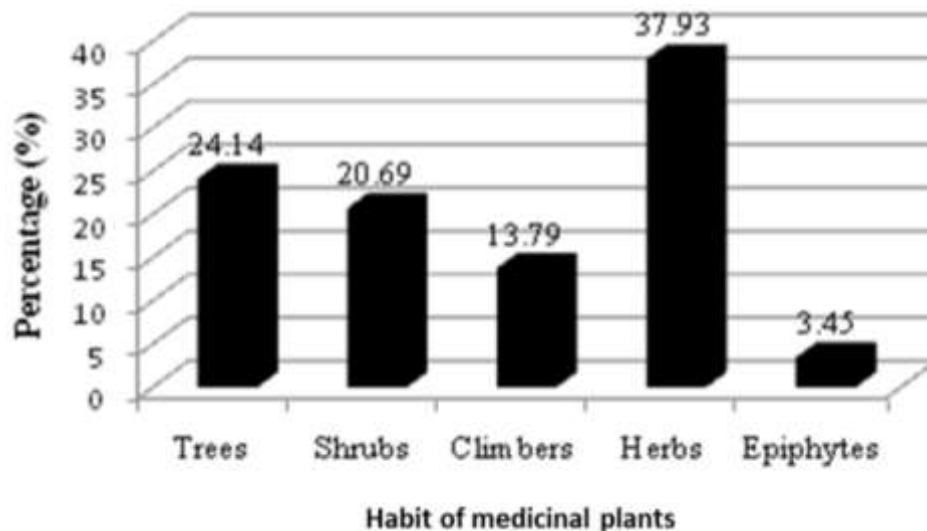
Figure 4. Habit of medicinal plants in the study area.

trend of using more of herbaceous plants could be advantageous as it is easier to cultivate them when they are in short supply and even naturally grown in natural environment. In similar fashion, the present study was in line with the report made by Mirutse et al. (2009) in Southwest Ethiopia, in cases where it is humid for most months of the year creating a favorable condition for the growth of herbs. Apparently, the herbs were the most harvested for the treatment of diseases of humans and

the livestock, which agrees with the previous studies of Mirutse (2001), Mirutse et al. (2009) and Mohammed and Berhanu (2011) on ethnobotanical conducted in Ethiopia. Nonetheless, the findings of Debela et al. (2004), Haile and Delenasaw (2007) and Fisseha et al. (2009) conducted in various parts of the country, sees shrubs as the primary growth form of remedies. Furthermore, the study area remains moisture/humid for several months for the year creating conducive environments for the

Figure 5. Habitat of medicinal plants in the study area.

growth and management of herbs. Thus, this is why local people of the study area mostly harvested leaves, fruits, seeds, rhizomes and bulbs of crops and herbs in every season.

Habitat of medicinal plants

Based on the data collected from key informants of the study area, around 8 species (27.59%) medicinal plants were obtained from both homegarden and 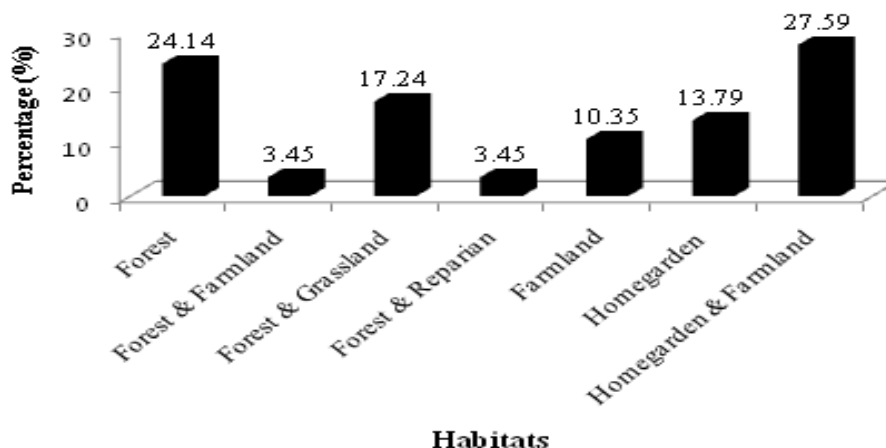farmland closely followed by 7 species (24.14%) obtained from forest, and 5 species (17.24%) collected from forest and grassland area (Figure 5). The finding of the present study agreed with the study conducted in other part of Ethiopia by Yalew et al. (2012), which revealed that medicinal plant (47.37%) were collected from the wild in the nearby forest and grassland areas, and almost above half (51.5%) of the medicinal plants were collected from the natural habitat (Gidey et al., 2011). On the other hand, the finding was in line with that of Gidey (2010) who reported highest number of medicinal plants from home garden in Alamata Town, Northern Ethiopia. This difference might be related to the variation in medicinal plant distribution in different climatic zone. Moreover, Yeki district got rain throughout the year unlike northern part of the country which gets sufficient rain in only two seasons mainly summer and autumn.

Plant parts used

Cumulatively, twenty nine (29) medicinal plants were used in the 4 sites (villages) of the district for the treatment of gastrointestinal parasitic infection/diseases in human. Frequently cited plant by the traditional healers was *Sonchus bipontini* (Asteraceae). The data revealed that most of the medicinal plants are used separately.

Different plant parts such as leaves, stem, barks, fruits, roots, seeds and flowers were used in preparing the remedies. The plants parts widely used by the traditional healers are the leaf (51.72%) followed by bark, seed, fruit and leaf and roots each with 6.89% (Figure 6). Grinding and mixing with water are the predominant modes of preparation of the medicine plants provided by local herbalists, and the oral route is used as the main administration way (96.55%). The dosage of a remedy depends on the age of the patient. In general, children have small quantities compared to adult. Almost all of the interviewed healers were men. Generally, they use their knowledge on medicinal plants to help the community by selling their products to the patients in the village and district. The major signs/symptoms diagnosis by healer to treat the parasite infection considered as being due to gastrointestinal parasites infections were stomach-ache, diarrhea, the presence of the worms and blood in the faeces. Five (20.83%) of the medicinal plants reported were approved, and commonly used in all of the four sites for the treatment of gastrointestinal parasitism.

Mode of remedy preparation and administration

Most of the parts of the plant used to treat gastrointestinal parasite was fresh form of the medicinal plants which are given to the patient for treating the parasite. The medicine extract which is chew directly or through swallowing was actively given to parasitic patient orally (96.55%) and dermal (3.45%) (Figure 7). The remedies were prepared, crushing a single plant (62.5%) and in few cases mixtures of different plants (37.5%). In the study, there were various methods of preparation and application for the different types of remedies identified. Also, various preparations forms were observed like infusion, crushing, decoction, concoction, powdering and chewing. However, there were few inconsistencies in the
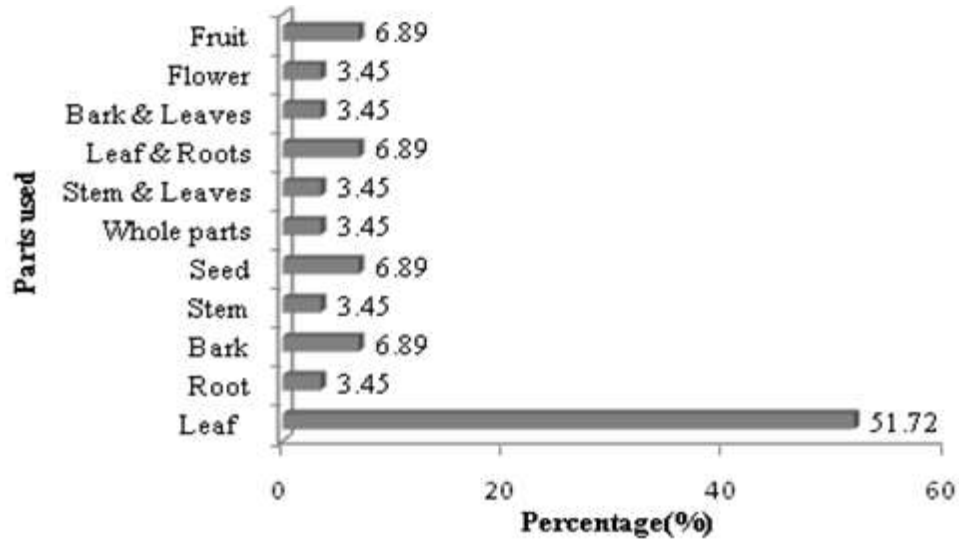
Figure 6. Parts of medicinal plants used for treatment of intestinal parasitic diseases.
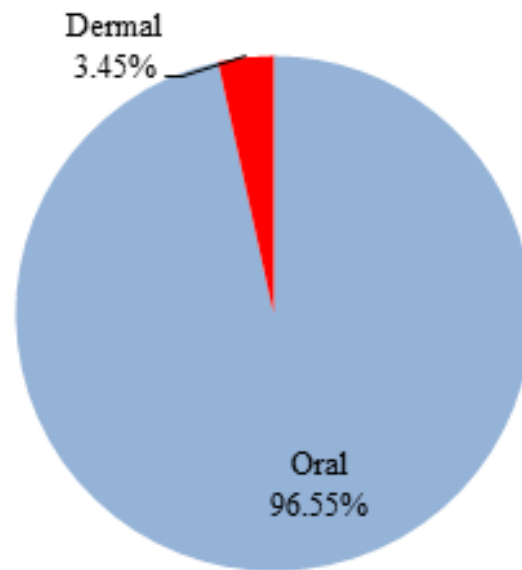
Figure 7. Route of administration to treat intestinal parasite.

parts used, the indications and the mode of preparation of the plants products from a site to each other. The plants parts were used separately but sometimes, traditional healers also used some ingredients such as honey, butter, cheese, milk and black barley to prepare the remedies (Table 3).

Conservation perspectives

As pointed out by informants in the study area, highest number of natural medicinal plants entails that majority of

the plants should not be cultivated purposively by traditional healers for the uses of remedies. In addition to this, the area is moist rich with plant diversity and possible to harvest numerous plants from wild immediately to treat the patient. Accordingly, local practitioners of the study area depend on the wild source of natural environment as compare to the ones cultivated (home gardens) to obtain the medicinal plants. But the activity of management mechanisms for future sustainability of wild medicinal plants is still ineffective. Based on the study, it needs emphasis to identify the threatened plants and to take appropriate conservation

Table 3. List of medicinal plants used for treatment of gastrointestinal parasitic diseases in Yeki districts, Southwest Ethiopia.

Species	Local name	Hb	Hab	PU	Diseases treat	Mode of preparation	RA	Voucher number
Aeollanthus densiflorus Ryding (Lamiaceae)	Yexxo (K)	H	FL, GL, HG	St and L	Amoeba	Grind the fresh leaf and mix the extract with milk and boil it, then drink1 glass a day for 2 days	Or	BDM007
Ajuga integrifolia (Lamiaceae)	Armagussa (Am)	H	FL, FR	L	Amoeba (chronic)	Grind them and drink 1 joint small finger size juice on empty stomach for 3 days	Or	BDM011
Brucea antidysenterica J.F. Mill. (Simaroubaceae)	Abalo (Am)	T	FR	L	Amoeba (Light)	Grind the leaf tip (bud) of Nukesho and Ye'ero then mix them with butter. Drink the juice with coffee cup measures depth of little finger joint every morning for two days. Detoxify by eating four spoon of cheese for each term	Or	BDM021
Canthium oligocarpum Hiern (Rubiaceae)	Afo(K)	Sh	FR	B	Amoebia diarrhea	Pounding, squeezing and mixing with honey	Or	BDM008
Celosia trigyna L. (Amaranthaceae)	Daggichoo (K)	H	HG	L and R	Tape worm	Chew the leaf (four mouthfuls) and swallow per a day for four days. No side effect. Eating Raw leaf 4 times in a day but not for pregnant. Boil root then next morning mix the extract with honey then take 1glass/1cup for baby	Or	BDM021
Cineraria abyssinica Sch. Bip. ex A. Rich. (Asteraceae)	Nopphoo (K)	Cl	F	L	Ascaris	Squeeze the leaf	Or	BDM012
Cleome gynandra L. (Capparidaceae)	Allaatti ate (K)	H	FL	L	Ascaris	Squeeze the leaf	Or	BDM025
Croton macrostachyus Del. (Euphorbiaceae)	Waagoo (K)	T	FR, FL	B	Ascaris	Grind and mix with porridge then eat it	Or	BDM022
Cucurbita pepo L. *(Cucurbitaceae)*	Duba (Am)	Cl	HG	Se	Intestinal worms	Boil the seed and eat it as food	Or	BDM005
Cyanoglassum lanceolatum Forssk. (Boranginaceae)	Caaqqo (Or)	H	FL	R	intestinal parasites	Ground and squeeze	Or	BDM003
Embelia schimperi Vatke (Myrsinaceae)	Enqoqo (Am)	T	FR	Fr	Tape worms	Crush the fruit in the form of juice and drink it for 1-2 days	Or	BDM006
Hagenia abyssinica (Bruce) J.F. Gmel. (Rosaceae)	Koso (Am)	T	FR	Fl	Tapeworm	Powdering from dry flowers and dissolve it with water then drunken	Or	BDM004
Justicia shimperina (Hochst. ex Nees) T.Anders (Acanthaceae)	Sensel (Am)	Sh	Hg and FL	L	Tapeworm	Ground and squeeze the leaf	Or	BDM016

Table 3. Contd.

Kalanchoe petitiana A. Rich (Crassulaceae)	Indahula (Am)	H	FR, GL	L	Ascaris	Putting fired leaf and put on the stomach of infants	Dr	BDM020
Carica papaya L. (Caricaceae)	Papaya (Am)	H	HG, FL	Se	Amoebia	Crush seed and mix with honey then drink the prepared juice	Or	BDM018
Maytenus arbutifolia (Celastraceae)	Atat(Am)	Sh	FR, GL, FL	L	Amoeba	Grind the mixture of leaves of Atato, tikur inchet and Ye'ero together. Then drink the wet extract with cup of 1st line of a small finger for 2 days	Or	BDM026
Microglossa pyrifolia (Lam.) Kuntz (Asteraceae)	Nibasho	Sh	GL, FL	L	Amoeba	Grind the fresh leaf and then mix the extraction with yogurt and drink the mixture four spoon per a day	Or	BDM001
Ocimum lamifolium Hochst. Ex Benth. (Lamiaceae)	Dama kese (Am)	Sh	FL,HG	L	Ascaris	Grind the leaf and mix the extraction with water. Then drink for babies ¼ of adult; No side effect Grind the fresh leaf and drink the extract with water.1glass a day for 2 days/in cup for baby, baby>5 year	Or	BDM015
Oncinotis tenuiloba Stapf. (Apocyanaceae)	Ceno (K)	Cl	FR, GL	L	Chronic amoeba (bloody)	Crush the mixture of fresh leaves of Ceno together with leaves tip of Ye'ero and then mix the extraction with cheese (with butter) or honey. Then eat four spoon or mouthful every morning for three days	Or	BDM028
Peperomia abyssinica Miq. (Piperaceae)	Gergio (Sh)	Ep.	FR, RP	St	Amoeba	Chew and swallow the juice of fresh stems	Or	BDM024
Peponium vogelii (Hook.f) Engl. (Cucurbitaceae)	Tojo (Sh)	Cl	GL, HG	Fr	Intestine parasites	Eat the fruit as he/she can possible. Drinking alcohol is forbidden	Or	BDM009
Pittosporum virdiflorum Sims. (Pittosporaceae)	Shello (Sh)	T	FR, GL	L	Intestinal parasite	Grind the fresh leaves and drink the extracted juice.	Or	BDM002
Prunus africana (Hook.f.) Kalkman (Rosaceae)	Tikur enchet (Am)	T	FR	L	Amoeba (Bloody diarrhea)	Crush the leaf tip of tikur inchet together with embuay then eat with ox beef or cheese (with butter) or honey for two to three days; for babies and pregnancy	Or	BDM027
Pycnostachys meyeri Gilrke (Lamiaceae)	Ye'ero (K)	H	GL, FR	L	Amoeba	Grind in wet, mix with water then drink1coffe cup for 4 days and ¼ for babies	Or	BDM019
Ricinus communis L. (Euphorbiaceae)	Gulo/ Kobo (Am)	Sh	HG, FL	L and R	Intestinal parasite	Crush the leaves together with the leaves of tikur inchet and then extract juice and prepare smooth ox meat and roast it until it dry enough. Then eat with smooth roasted ox beef/meat one per a day two days; drinking water is forbidden	Or	BDM017
Ruta chalepensis L. (Rutaceae)	Caddiraammoo	H	Hg	WP	amoebic dysentery	Ground and squeeze	Or	BDM013

Table 3. Contd.

Solanum anguivi Lam. (Solanaceae)	Embuay (Am)	H	FL, HG, GL	L		Amoeba	Grind the leaf and drink the extraction or crush the mixture tikur inchet then eat by mixing with butter, abish, and doro meat Is better to take alcohols As food at morning; No side effect;	Or	BDM023
Sonchus bipontini Asch. (Asteraceae)	Meracho (Sh)	H	FL, HG,	L		Amoeba	The fresh leaf of the plant mix with honey and/ or milk (cheese). Then the mixture is eaten two mouthfuls or spoon per a day. This also has been given for pregnancy; No side effect because the cheese/milk is as detoxifier; For children ¼ of the adult. Grind the leaf of Meracho and Nukesho and then mix the extraction with water. Drink the juice with coffee cup measure depth of small finger of upper joint; Detoxified by eating two mouthful or spoon of honey or cheese/ergo; no side effect; forbidden for pregnancy.	Or	BDM029
Vangueria madagascariensis Gmel. (Rubiaceae)	Gujimato (Sh)	T	FR	L and B		Ascaris	Grind the leaf of Gujimato and prepare porridge with bula and then eat the porridge as a food. 1.Boil and drink the extract for 4 days 1glass for adult and 1 coffee cup for babies; 2.mix with barely powder then drink	Or	BDM010

NB:- Hb=Habit (T=Tree, Sh=Shrub, H=Herb, Cl=Climber, Ep.=epiphyte); Hab=Habitat (FR= Forest, FL=farmland, HG=homegarden, RP= riparian); PU=part use (L=Leaves, St=stem, R=Roots, B=bark, Fr= Fruit, Se=Seed, Fl=flower, Fr=fruit, WP=whole part); RA=Route of administration (Or=Oral, Dr=Dermal).

measures in the district. According to Lulekal et al. (2008) and Yalew et al. (2012), local practitioners depend on the wild source of medicinal plants. Hence, the activity of managing and conserving medicinal plants in natural environment is not easy if the plants are over exploited then there will be a challenge in the conservation of resources in their wild environments. Various ethnomedicinal studies conducted in different regions proved the presence of great diversity of medicinal plants and occurrence of gastrointestinal infections (Tariq et al., 2015).

Conclusion

This study underlined that 29 medicinal plants were used to treat gastrointestinal parasites diseases in human, and the symptoms that might be related to gastrointestinal parasites identified by traditional healers. Therefore, there is a realistic potential that these plants would contain compounds with anti-parasitic potency. This study contributes to the enormous indigenous knowledge on medicinal plants and plant-based remedies practiced within ethnic groups. The finding needs the improvement and promotion of the local traditional practitioners using medicinal plants to treat gastrointestinal parasites. There is also the need to investigate the phytochemical, pharmacological and toxicological profile of the plants used in order to ensure their efficacy as well as their safety. Anti-parasitic tests should be carried out to assess the efficacy of the inventoried plants on gastrointestinal parasites. Every stakeholder must consider the status of these very valuable medicinal plants to propagate in homegarden and botanical garden for Ex situ conservation purposes.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

knowledge as well as the elders and the local administration for their support in facilitating the interview process. We also thank the staff of Biology Department and Mizan-Tepi University for their support, and the National herbarium of Addis Ababa University for letting them make use of their Herbarium during plant identification

REFERENCES

Abdullah I, Tak H, Ahmad F, Gul N, Nabi S, Sofi TA (2016). Predominance of Gastrointestinal Protozoan Parasites in Children: A Brief Review. Journal of Health Education Research and Development 4:4.

Al Saqur IM, Al-Warid HS, Albahadely HS (2017). The prevalence of Giardia lamblia and Entamoeba histolytica/ dispar among Iraqi provinces. Karbala International Journal of Modern Science 3:93-96.

Corrigan BM, Van Wyk BE, Geldenhuys CJ, Jardine J (2011). Ethnobotanical plant uses in the KwaNibela Peninsula, St Lucia, South Africa. South African Journal of Botany 77:346-359.

Central Statistics Agency (CSA) (2007). Summary and statistical report of population and housing census, Addis Ababa, Ethiopia.

Debela H, Zemede A, Ensermu K (2004). Use and management of ethnoveterinary medicinal plants by indigenous people in Boosat, Welenchiti area. Ethiopian Journal of Biological Sciences 3(2):113-132. Available at: https://www.ajol.info/index.php/ejbs/article/view/39007

Fisseha M, Sebsebe D, Tilahun T (2009). An Ethnobotanical study of medicinal plants in Wonago Woreda, SNNPR, Ethiopia. Journal of Ethnobiology and Ethnomedicine 5(28):1-18.

Gidey Y (2010). Ethnobotanical study of medicinal plants in and around Alamata, Southern Tigray, Northern Ethiopia. Current Research Journal of Biological Sciences 2(5):338-344.

Gidey Y, Mekonen T, Mezgebe K (2011). Survey of medicinal plants used to treat human ailments in Hawzen district, Northern Ethiopia. International Journal of Biodiversity and Conservation 3(13):709-714.

Haile Y, Delenasaw Y (2007). Traditional medicinal plant knowledge and use by local healers in Sekoru District, Jimma Zone, Southwestern Ethiopia. Journal of Ethnobiology and Ethnomedicine 3(24):1-7.

Haque R (2007). Human Intestinal Parasites. Journal of health, population and nutrition 25(4):387-391.

Kefalew A, Asfaw Z, Kelbessa E (2015). Ethnobotany of medicinal plants in Ada'a District, East Shewa Zone of Oromia Regional State, Ethiopia. Journal of Ethnobiology and Ethnomedicine 11:25.

Lulekal E, Kelbessa E, Bekele T, Yineger H (2008). An ethnobotanical study of medicinal plants in Mana Angetu District, southeastern Ethiopia. Journal of Ethnobiology and Ethnomedicine 4:10.

Mirutse G (2001). An Ethnobotanical study of medicinal plants used by the Zay People in Ethiopia. CBM: Slentserie 3:81-99.

Mirutse G, Zemede A, Zerihun W, Tilahun T (2009). Medicinal plant knowledge of the Bench ethnic group of Ethiopia: an ethnobotanical investigation. Journal of Ethnobiology and Ethnomedicine 5(34):1-35.

Mohammed A, Berhanu A (2011). Ethnobotanical survey of traditional medicinal plants in Tehuledere District, South Wollo, Ethiopia. Journal of Medicinal Plants Research 5(26):6233-6242.

Ngarivhume T, van't Klooster Cl, de Jong JT, Van der Westhuizen JH (2015). Medicinal plants used by traditional healers for the treatment of malaria in the Chipinge district in Zimbabwe. Journal of Ethnopharmacology 159:224-237.

Norhayati M, Fatmah MS, Yusof S, Edariah AB (2003). Intestinal Parasitic Infections in Man: A Review. Medical Journal of Malaysia 58(2):296-300.

Pankhurst R (2001). The status and availability of oral and written knowledge on traditional health care. In: Conservation and Sustainable Use of Medicinal Plants in Ethiopia Proceeding of The National Workshop on Biodiversity Conservation and Sustainable Use of Medicinal Plants in Ethiopia, (Medhin Zewdu and Abebe Demissie eds.). IBCR, 28 April-01 May 1998, pp. 92-106.

Speich B, Marti H, Ame S M Ali, SM, Bogoch II, Utzinger J, Albonico M, Keiser J (2013). Prevalence of intestinal protozoa infection among school-aged children on Pemba Island, Tanzania, and effect of single-dose albendazole,nitazoxanide and albendazole-nitazoxanide. Parasites and Vectors 6:3.

Stepek G, Buttle DJ, Duce IR, Behnke JM (2006). Human gastrointestinal nematode infections: are new control methods required? International journal of experimental pathology 87(5):325-341.

Tariq A, Mussarat S, Adnan M, Abd-Allah EF, Hashem A, Alqarawi AA, Ullah R (2015). Ethnomedicinal Evaluation of Medicinal Plants Used against Gastrointestinal Complaints. BioMed Research International P 14.

Tolossa K, Debela E, Athanasiadou S, Tolera A, Ganga G, Houdijk JGM (2013). Ethno-medicinal study of plants used for treatment of human and livestock ailments by traditional healers in South Omo, Southern Ethiopia. Journal of Ethnobiology and Ethnomedicine 9:32.

Traore A, Ouedraogo S, Lompo M, Traore S, Some N, Guissou IP (2013). Ethnobotanical survey of medicinal plants used to treat gastrointestinal parasites in human and livestock in four geographic areas of Burkina Faso (West Africa). Archives of Applied Science Research 5(6):172-177.

Ukwubile CA (2012). Anti-Helminthic Properties of Some Nigerian Medicinal Plants on Selected Intestinal Worms in Children (Age 5-13) in Ogurugu, South East Nigeria. Bacteriology and Parasitology 3:159.

Utzinger J, N'Goran EK, Marti HP, Tanner M, Lengeler C (1999). Intestinal amoebiasis, giardiasis and geohelminthiases: their association with other intestinal parasites and reported intestinal symptoms. Transactions of the Royal Society of Tropical Medicine and Hygiene 93(2):137-41.

Yalew A, Debebe Y, Ashok KP, Zewdneh T, Assefa A (2012). Traditional medicinal plants used by people in Libo-Kemkem District, South Gondar, Ethiopia. Asian Journal of Agricultural Sciences 4(3):171-176.

Yeki Woreda Administration office (2014). Yeki Woreda Administration office (2014). Annual report of Yeki Woreda Agricultural and Rural development Office, Unpublished Data.

Related Journals:

Clinical Reviews and Opinions

Journal of
Medicinal Plant Research

African Journal of
Pharmacy and Pharmacology

Journal of
Dentistry and Oral Hygiene

Journal of
Parasitology and Vector Biology

Journal of
Pharmacognosy and Phytotherapy

Journal of
Medical Laboratory and Diagnosis

Journal of
Diabetes and Endocrinology

Medical Practice and Reviews

www.academicjournals.org